Notice of Allowability	Application No.	Applicant(s)	
	09/786,009	XU ET AL.	
	Examiner	Art Unit	
	William W. Moore	1652	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to *the amendment filed 01 June, and the interview conducted 17 August, 2004*.

2. ☒ The allowed claim(s) is/are *12-18,21-23 and 25-30*.

3. ☒ The drawings filed on *01 June 2004* are accepted by the Examiner.

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
   a) ☐ All b) ☐ Some* c) ☐ None of the:
      1. ☐ Certified copies of the priority documents have been received.
      2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
      3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

   * Certified copies not received: _____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.

6. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
   (a) ☐ including changes required by the Notice of Draftsperson's Patent Drawing Review (PTO-948) attached
      1) ☐ hereto or 2) ☐ to Paper No./Mail Date _____.
   (b) ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date _____.

   Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

7. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892)
2. ☐ Notice of Draftperson's Patent Drawing Review (PTO-948)
3. ☐ Information Disclosure Statements (PTO-1449 or PTO/SB/08), Paper No./Mail Date _____
4. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material
5. ☐ Notice of Informal Patent Application (PTO-152)
6. ☒ Interview Summary (PTO-413), Paper No./Mail Date *20040817* .
7. ☒ Examiner's Amendment/Comment
8. ☒ Examiner's Statement of Reasons for Allowance
9. ☐ Other _____.

EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Amend claims 12, 17, 22, 25, 28, and 29 thus:

12. (Amended) A method for preparing a target protein with a carboxy-terminal thioester, comprising:

(a) expressing ~~in a host cell,~~ a recombinant precursor protein in a host cell, the precursor protein comprising the target protein fused at its carboxy terminus to ~~an intein, the intein having an~~ the amino terminus of an intein, the intein ~~having an amino terminus and a carboxy terminus wherein the amino terminus is fused to the target protein and the carboxy terminus and is optionally fused to a binding protein domain, the intein~~ being selected from the group consisting of a native intein, an intein derivative, and ~~or~~ a mutant intein ~~mutant~~, wherein the intein is optionally fused at its carboxy terminus to a binding protein domain; and,

(b) contacting the expressed precursor protein with 2-mercaptoethanesulfonic acid to induce ~~and inducing~~ cleavage of the intein from the precursor protein; thereby forming ~~so as to form~~ the target protein having the carboxy-terminal thioester.

17. (Amended) A method for expressing a recombinant protein precursor, comprising:

(a) inserting a nucleic acid sequence encoding a target protein into a plasmid at a multiple cloning site located upstream of and in frame with a fusion gene encoding an intein and a binding protein domain, wherein

(i) the intein is selected from the group consisting of a native ~~naturally-occurring~~ intein, an intein derivative, or a ~~an~~ mutant intein ~~mutant~~; and

(ii) the multiple cloning site contains a linker having a nucleic acid sequence ~~and the linker is~~ selected from the group consisting of SEQ ID NO:1; SEQ ID NO:2; SEQ ID NO:3; and ~~or~~ SEQ ID NO:4; and

(b) transforming a host cell with ~~introducing~~ the plasmid ~~into a host cell~~ and providing conditions suitable for expressing the recombinant precursor protein by the host cell;

whereby the recombinant protein precursor is expressed.

22. (Amended) A method of modifying a target protein by ligating a chemically synthesized peptide or protein ~~*in vitro*~~ to the target protein *in vitro*, comprising:

(a) expressing ~~in a host cell,~~ a recombinant precursor protein in a host cell, the precursor protein comprising the target protein fused at its carboxy terminus to the amino terminus of an intein selected from the group consisting of~~:~~ a ~~an~~ native intein, an intein derivative, and ~~or~~ a mutant intein, wherein the intein is optionally fused to a binding protein domain at its carboxy terminus~~, wherein the intein is capable of thiol-induced cleavage~~;

(b) inducing cleavage of the intein from the target protein by ~~adding~~ contacting the precursor protein with 2-mercaptoethanesulfonic acid thereby ~~so as to~~ forming ~~form~~ a carboxy-terminal thioester on the target protein;

(c) obtaining the chemically synthesized peptide or protein having an amino terminal cysteine; and,

(d) ligating the target protein of step (b) to the chemically synthesized peptide or protein of step (c);

thereby forming ~~to form~~ a modified target protein.

25. (Amended) A method of labeling a target protein~~,~~ comprising:

(a) expressing a recombinant precursor protein in a host cell, the precursor protein comprising the target protein fused at its carboxy terminus to the amino terminus of an intein, the intein ~~having an amino terminus and a carboxy terminus wherein the amino terminus is fused to the target protein and optionally fused to a binding protein domain at the carboxy terminus the intein~~ being selected from the group consisting of a native ~~naturally occurring~~ intein, an intein derivative, and ~~or~~ a mutant intein ~~mutant~~, wherein the intein is optionally fused at its carboxy terminus to a binding protein domain ~~wherein the intein is capable of thiol induced cleavage~~;

(b) inducing cleavage ~~cleaving~~ of the intein from the target protein by contacting the precursor protein with ~~in the presence of~~ 2-mercaptoethanesulfonic acid thereby ~~so as to~~ forming ~~form the target protein having~~ a carboxy-terminal thioester on the target protein;

(c) obtaining a chemically synthesized peptide or protein having a marker and an amino-terminal cysteine; and,

(d) ligating the target protein of step (b) to ~~with~~ the chemically synthesized peptide or protein of step (c) ~~for labeling~~;

thereby forming the labeled target protein.

28. (Amended) A method of restoring a biological activity to a polypeptide inactive due to the absence of a carboxyl proximal amino acid sequence region by ~~for~~ ligating a chemically synthesized protein or peptide comprising a carboxyl proximal amino acid sequence region of the polypeptide ~~to an inactive form of a protein so as restore~~ to the polypeptide lacking said region ~~protein activity~~, comprising:

(a) expressing a recombinant fusion protein in a host cell, the ~~a~~ fusion protein comprising the inactive form of the polypeptide ~~protein~~ fused at its carboxy terminus to the amino terminus ~~one~~ of an intein, the intein being selected from

the group consisting of a native intein, an intein derivative, and ~~or~~ a mutant intein ~~mutant~~, wherein the fusion protein is expressed from a plasmid;

(b) inducing cleavage of the intein ~~mediated cleavage~~ from the inactive form of the polypeptide by contacting the fusion protein of step (a) with ~~by adding~~ 2-mercaptoethanesulfonic acid thereby ~~so as to form~~ forming a carboxy-terminal thioester on the inactive form of the polypeptide ~~protein~~;

(c) obtaining a chemically synthesized peptide or protein having an amino-terminal cysteine; and,

(d) ligating the inactive form of the polypeptide having a carboxy-terminal thioester ~~protein~~ produced in ~~of~~ step (b) to the chemically synthesized peptide or protein of step (c) ~~to restore protein activity~~;

thereby restoring a biological activity of the polypeptide.

29. (Amended) The method according to claim 28, wherein the polypeptide ~~protein~~ to which ligation with a carboxyl proximal amino acid sequence region restores a biological activity is a cytotoxic protein.

Authorization for this examiner's amendment was given in a telephone interview with Dr. Harriet Strimpel on August 17, 2004.

The following is an examiner's statement of reasons for allowance:

Applicant's amended Figure 1 is accepted. Amendments to pages 2, 7, and 8 of the specification filed June 1, 2004, introduce no new matter because they correct obvious errors and incorporate the text of a U.S. Patent. The entry of amendments to claims 12-18, 22, 23, 25, 27, and 28, and the amendment to page 2 of the specification filed June 1, 2004, as well as the cancellation of claim 24, remove bases for an objection of record of claim 12 and a rejection of record of claims herein under the second paragraph of 35 U.S.C. § 112, and, together with Applicant's arguments filed June 1, 2004, also remove the basis for the rejection of record of claims 28-30 under the first paragraph of 35

U.S.C. § 112 for lack of enablement in restoring a protein activity. An amendment to claim 73 in the copending application serial No. 09/249,543 renders the provisional double-patenting rejection of record herein moot and the rejection is withdrawn.

The examiner's amendment above clarifies the recitations of intein components in claims 12, 17, 22, 25, and 28 to provide consistency throughout, ensuring both (i) that clauses (a) and (b) of claims 12, 22, 25, and 28 uniformly recite the salient features of recombinant precursor proteins, their components, and the cleavage process, and (ii) that claims 12, 17, 22, 25, and 28 uniformly state a proper Markush format for the three kinds of inteins useful in the claimed methods. The examiner's amendment also partitions the terminal clauses of claims 12, 17, 22, 25, and 28 to separate the final step from a terminal clause setting forth the result foreseen in the preamble, thus completing each claimed method. The examiner's amendment removes the superfluous recitations in claims 22 and 25 of "wherein the intein is capable of thiol induced cleavage" because, for the reasons set forth below, Applicant's arguments filed June 1, 2004, are persuasive in overcoming the rejection of record of claims 12-18, 22, 23 and 25-30 for lack of enablement under the first paragraph of 35 U.S.C. § 112, of claimed ligation methods utilizing native, i.e., unmodified, inteins. Further, the examiner's amendment restates claims 28 and 29 by adopting a term, "polypeptide inactive due to the absence of a carboxyl proximal amino acid sequence region", that is consistent both with the term, "truncated, inactive", found at page 3 of the specification, and the nature of a truncation indicated in Example 6 at page 21 of the specification, whereby it is understood that a recombinant fragment with a carboxyl-terminal thioester is the target protein, regardless of its size. The restatement of claims 28 and 29 in the examiner's amendment also does not require that a carboxyl proximal amino acid sequence region that corresponds exactly to an absent region be ligated to the target protein to restore activity in a claimed method.

Applicant's arguments are persuasive in overcoming the rejection of record of claims 12-18, 22, 23 and 25-30 for lack of enablement under the first paragraph of 35 U.S.C. § 112, of claimed ligation methods utilizing a native intein because the amendment to page 2 of the specification that specifically incorporates a portion of the disclosure of U.S. Patent No. 5,834,247 shows that one of skill in the art of using the properties of inteins and their flanking amino acid sequence elements to alter protein structure would consider a "native" intein to be an intein as it occurs in Nature before any modification by a person and would also consider that a "modified intein" had been a native intein before modification. Thus the term "any CIVPS" at page 8 of the instant specification is considered to refer to a splicing element of the '247 patent, and supports recitations of the term "native intein" in claim 12 as amended June 1, 2004, as well as in claims 17, 22, 25 and 28 as amended hereinabove and, most importantly, makes Applicant's citation of Telenti et al., 1997, of record, at page 16 of the Response filed June 1, 2004, dispositive of the issue of enablement.

Telenti et al. show, in Table 1, that recombinantly expressing of a fusion polypeptide wherein a wild-type, or native, *Mycobacterium xenopi* GyrA intein is flanked at its amino-terminus by a native GyrA extein region of 65 amino acids intervening between the intein and an amino-proximal maltose-binding protein and subsequently exposing the fusion polypeptide to a thiol reagent invariably results in the excision of the unmodified intein and the splicing of its flanking amino acid sequence elements in the fusion polypeptide. But Telenti et al. also show, in the first two blocks of comparisons across the top of Table 1, that recombinant expression of a fusion polypeptide wherein the amino-terminus of the unmodified GyrA intein is directly fused to the carboxyl terminus of the maltose-binding protein, exposure to the thiol reagent overwhelmingly induces cleavage, not splicing, at the amino-terminus of the intein, thus freeing the amino proximal maltose-binding protein, which is analogous to the acceptor protein of methods

of claims 12-18, 22, 23 and 25-30 herein. While Telenti et al. are silent as to the presence of a carboxyl-terminal thioester on the maltose-binding protein released by thiol reagent-induced cleavage; there is no reason to doubt that is present. This is because both Chong et al., 1997, and U.S. Patent No. 5,834,247, of record and also cited by Applicant in the Response filed June 1, 2004, used DTT and other thiol reagents to cleave junctions between the carboxyl-termini of acceptor proteins and the amino termini inteins, thereby generating carboxyl-terminal thioesters on the resulting acceptor polypeptides. Thus an artisan seeking to practice a claimed method requiring recombinant expression of a precursor protein and subsequent formation of a target protein having a carboxyl-terminal thioester, that is then available for ligation with a protein or peptide having an amino terminal cysteine, would be aware that direct fusion of the amino terminus of the native *Mycobacterium xenopi* GyrA intein to the carboxyl terminus of a desired target protein, or acceptor protein, in the absence of any intervening amino-proximal native GyrA extein amino acid sequence region would permit subsequent contact of the fusion polypeptide with a thiol reagent of the claims to produce carboxyl-terminal thioesters on a target, or acceptor, polypeptide cleaved from the carboxyl-proximal native intein.

Applicant's arguments are likewise persuasive in overcoming rejections of record of claims herein under 35 U.S.C. § 103 relying on a combination of teachings concerning MESNA of both Burton et al., US 5,789,578 and the Sixth Edition of The Dictionary of Organic Compounds with the teachings of ligation methods utilizing thiol reagents to cleave acceptor proteins from inteins of any of Chong et al., 1997, Severinov et al, 1998, or Muir et al. Applicant points out that the single mention of MESNA, among many other thiol reagents, at col. 6 of Burton et al. and the information that one of ordinary skill in the art at the time the invention was made might have obtained about MESNA from The Dictionary of Organic Compounds, taken separately or together, are

insufficient to motivate such an artisan to select MESNA as a substitute for any of the thiol reagents used by Chong et al., Severinov et al., and Muir et al., particularly where methods taught by Burton et al. did not concern cleavage of polypeptides to provide a thioester at the carboxyl terminus of a polypeptide or peptide or even to provide a thioester at the carboxyl terminus of a polypeptide or peptide absent any cleavage.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

Conclusion

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to William W. Moore whose telephone number is now 571.272.0933. The examiner can normally be reached between 9:00AM and 5:30PM EST. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Ponnathapura Achutamurthy, can now be reached at 571.272.0928. The fax phone numbers for all communications for the organization where this application or proceeding is assigned remains 703.872.9306. Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is now 571.272.1600.

William W. Moore
August 17, 2004